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Invited review

Paradigm shifts in understanding equine laminitis

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ABSTRACT

Laminitis, one of the most debilitating conditions of all equids, is now known to be the result of several systemic disease entities. This finding, together with other recent developments in the field of laminitis research, have provoked a rethink of our clinical and research strategies for this condition. First, laminitis is now considered to be a clinical syndrome associated with systemic disease (endocrine disease, sepsis or systemic inflammatory response syndrome, SIRS) or altered weight bearing rather than being a discrete disease entity. Next, laminitis associated with endocrine disease (endocrinopathic laminitis) is now believed to be the predominant form in animals presenting (primarily) for lameness. Third, the designation of laminitis as a primary and severe basement membrane pathology now requires revision. Instead, current data now proposes a variable subclinical phase associated with gross changes in the hoof capsule, with stretching and elongation of the lamellar cells an early and key event in the pathophysiology. These findings have fuelled new mechanistic hypotheses and research directions that will be discussed, together with their implications for future clinical management.

Introduction

Laminitis has long been recognised as a painful condition of the hoof that causes lameness in horses (Heymering, 2010), with modern studies of the condition that can be traced back to a doctoral thesis published in the late forties (Obel, 1948). Laminitis can have debilitating long-term effects as a recurrent or chronic condition and, at worst, necessitates euthanasia of an animal in considerable pain (Hunt, 1993). At clinical presentation, we typically see lameness involving one or multiple hooves, stiffness, weight shifting, a typical 'saw horse' stance and reluctance to move, increased digital arterial pulses and sensitivity to hoof tester pressure applied at the toe of the affected digit(s) (Dyson, 2011). Many horses also present with gross hoof wall alterations that include divergent rings, increased cap horn or a wider/separated white line, and flat or convex soles (Pollitt, 2004; Collins et al., 2010; Karikoski et al., 2015) (Fig. 1).

Historically, 'classical laminitis' was linked with severe conditions associated with sepsis or systemic inflammatory response syndrome (SIRS), with deliberate starch overload or with the induction of metritis used to create experimental models (Obel, 1948). Conversely, supporting limb laminitis can result from any unilateral, painful lameness with prolonged abnormal weight bearing (Baxter and Morrison, 2009; van Eps et al., 2010; Virgin et al., 2011). The 1980s saw publication of the first hypotheses to link endocrine disease and laminitis, termed 'endocrinopathic laminitis' (Coffman and Colles, 1983; Jeffcott et al., 1986), with resistance to insulin or its abnormal regulation being a common association, especially in pony breeds.

Until relatively recently, research continued to focus on laminitis provoked by inflammation. It took a ground breaking study of a single herd of mixed Welsh and Dartmoor breed ponies, some with previous laminitis, to reveal the differences between these and 'laminitis-naive animals' (Treiber et al., 2006). Critically, these differences included higher basal insulin concentrations, plasma triglycerides, and body condition scores (Treiber et al., 2006). The five-fold increase in basal insulin concentration that accompanied the redevelopment of laminitis in a subset of ponies ($n = 13$) grazed on lush pasture was a particularly provocative finding (Treiber et al., 2006). Contemporaneously, an independent group successfully developed an experimental model of hyperinsulinaemic laminitis (Asplin et al., 2007) based on clinical observations made with horses with pituitary pars intermedia dysfunction (PPID) or other endocrine diseases (now termed equine metabolic syndrome, EMS). The high frequency of horses demonstrating (marked) hyperinsulinaemia when affected with endocrine disease was already known, together with the poorer prognosis (survival over 2 years) that accompanies presentation

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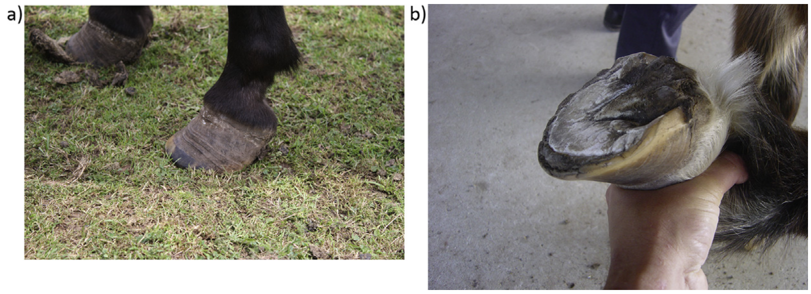

Fig. 1. (a) Divergent rings on the outer hoof wall of a pony without current lameness. (b) Solar hoof surface of a pony showing a convex or 'dropped' sole and a widened white line.

with hyperinsulinaemia (>188 μIU/mL) vs. normal or moderately increased levels of basal serum insulin (<62 μIU/mL) (McGowan et al., 2004).

More recently, studies of spontaneous endocrinopathic laminitis and hyperinsulinaemic models have markedly changed our perception of laminitis, which now warrants a re-evaluation of its clinical management and future research directions.

Paradigm shift 1: Laminitis is a clinical syndrome

Laminitis is now thought to be a clinical syndrome (rather than a discrete disease) that results from several systemic disease entities or, less frequently (as above), in the supporting limb of a lame horse (Baxter and Morrison, 2009; van Eps et al., 2010; Virgin et al., 2011; Wylie et al., 2015). Since 1948, laminitis had been considered to be a disease entity, albeit with obscure cause(s) that could be studied in the context of SIRS induced with starch or oligofructose, collectively termed carbohydrate overload models (Obel, 1948; Garner et al., 1975; Galey et al., 1991; van Eps and Pollitt, 2009). Despite recognition of the association between endocrine disease and laminitis, this connection was largely ignored. It was not until 'endocrinopathic' laminitis was unequivocally induced in a hyperinsulinaemia model that it became clear that laminitis could be provoked by multiple stimuli. Specifically, laminitis was induced in 5/5 previously healthy, young, and lean ponies following exposure to prolonged hyperinsulinaemia while maintaining euglycaemia (Asplin et al., 2007). Repetition in normal Standardbred horses yielded similar results (de Laat et al., 2010), while mild hyperglycaemia (mean ± standard error, SE, glucose 10.7 ± 0.78 mmol/L) and endogenous hyperinsulinaemia (mean ± SE insulin 208 ± 26.1 μIU/mL) induced lamellar lesions, but not a painful condition (de Laat et al., 2012).

This simple but important paradigm shift had several implications, the main one being that an accurate diagnosis of the associated systemic disease (or abnormal weight bearing) would be pivotal for laminitis management, prognosis and the prevention of recurrence. Additionally, the recognition of divergent causes of laminitis and associated risk factors (Table 1) could now improve the design of research studies that had, hitherto, proven to be inconclusive (Wylie et al., 2012).

Paradigm shift 2: Endocrinopathic laminitis predominates in animals presenting for lameness

Endocrinopathic laminitis is now recognised as the most common form of naturally occurring laminitis in horses and ponies presenting primarily with lameness in developed countries, including the USA and Europe (Donaldson et al., 2004; Karikoski et al., 2011). An earlier misconception that laminitis was predominantly associated with sepsis or SIRS arose from its prevalence in equids treated at veterinary referral hospitals, where laminitis research is concentrated (Parsons et al., 2007). This misperception was highlighted by a large epidemiological study in the USA (USDA, 2000), which showed that grain overload, retained placenta, colic or diarrhoea accounted for only 12% of owner-reported cases of laminitis; the remainder were associated with dietary problems or obesity, or were of unknown cause. Subsequent, more convincing studies from the USA and Europe

Table 1
Comparison of key signalment and clinical findings in horses ($n = 73$) developing laminitis during hospitalisation (Parsons et al., 2007) compared to those ($n = 36$) presenting with lameness due to laminitis as the primary problem (Karikoski et al., 2011).

Factor	Hospitalised horses (Parsons et al., 2007)	Primary laminitis (Karikoski et al., 2011)
Mean age	5.8 years	15 years Laminitic horses significantly older than hospital controls (9 years; $P < 0.001$)
Breeds	82.5% light breeds (Thoroughbred, Standardbred, Quarterhorse and Arabian)	86% pony, coldblood or warmblood Ponies were significantly overrepresented compared to hospital controls ($P = 0.002$)
Endotoxaemia (SIRS)[a]	Present, and significantly associated with the risk of development of laminitis on multivariable analysis (odds ratio 5, 95% confidence interval 1.37-18.19)	Not present[b]
Other indicators of illness	Yes, on univariable analysis	None
Phenotypic indicators of equine metabolic syndrome/diagnosis of pituitary pars intermedia dysfunction (PPID)	Not recorded	58%/34%
Hyperinsulinaemia	Not recorded	97%
Evidence of previous laminitis	Not recorded	86%

[a] Endotoxaemia, now termed systemic inflammatory response syndrome (SIRS), was diagnosed on the basis of clinical signs, such as hyperaemic mucous membranes and hyperaemic perialveolar gingiva, and supportive laboratory findings, such as neutropaenia and toxic changes in neutrophils (Parsons et al., 2007).
[b] Clinical evidence of SIRS was an exclusion criterion for the study (Karikoski et al., 2011).

identified endocrinopathies in 90% of cases of laminitis in horses/ponies presenting for lameness (Donaldson et al., 2004; Karikoski et al., 2011).

The major endocrinopathic disorders resulting in laminitis are EMS and/or PPID, the former characterised by obesity, insulin resistance/dysregulation and laminitis (Frank et al., 2010; Frank and Tadros, 2014). PPID is a disease of aged horses in which a loss of dopaminergic inhibition of the pituitary pars intermedia allows overproduction of pituitary hormones (including ACTH, α-MSH, CLIP and β-endorphin) (McFarlane, 2011). The clinical signs of PPID include hypertrichosis and abnormal hair shedding patterns, muscle wastage, abnormal fat distribution, polyuria and polydipsia, susceptibility to infection and infertility (McFarlane, 2011). PPID has also been suggested to cause both hyperinsulinaemia and associated laminitis (Durham et al., 2014), possibly in a subset of horses with PPID. Field based epidemiological studies have shown that the prevalence of hyperinsulinaemia (32%) and laminitis (13%) is greater in horses with PPID than in age-matched controls (McGowan et al., 2013). Higher values for the prevalence of laminitis are quoted in case series (24–82%) that suffer bias from owner selection for veterinary presentation (Schott, 2002). EMS, or at least hyperinsulinaemia, is emerging as the predominant endocrine cause of laminitis (Karikoski et al. 2011). Furthermore, in a recent histological study of lamellar pathology, all horses and ponies with PPID and lamellar pathology had fasting hyperinsulinaemia, whereas PPID animals without lamellar pathology manifested normal serum insulin concentrations (<20 mIU/L; Karikoski et al., 2016).

Paradigm shift 3: The pathology of laminitis: Endocrinopathic versus SIRS-associated laminitis; different or a matter of degree?

The final paradigm shift involves changes in our understanding of the pathology of laminitis, with the identification of lamellar cell stretch as an early and potentially crucial lesion (Karikoski et al., 2014, 2015, 2016).

The availability of the hyperinsulinaemia model provided an opportunity to compare hoof pathology to previous descriptions based on SIRS-associated laminitis (Obel, 1948; Garner et al., 1975; Roberts et al., 1980; Pollitt, 1996). While diseases associated with SIRS are precipitous and frequently severe, endocrine disease is typically chronic, with a potentially protracted subclinical phase. However, exploration of the histology of the lamellar region in either scenario revealed a relatively conservative spectrum of lesions (Asplin et al., 2007; de Laat et al., 2011b, 2013a; Karikoski et al., 2014, 2015, 2016), possibly reflecting a limited functional plasticity for this highly specialised tissue or mechanistic similarities in its injury. With the caveat that models of hyperinsulinaemia manifest an abrupt rather than (more natural) insidious onset of high insulin levels (in otherwise clinically normal individuals), these models clearly demonstrate that hyperinsulinaemia can cause laminitis, and indicates that lamellar structures are an early target (6–48 h; de Laat et al., 2013a). Comparable tissue from naturally occurring cases is unavailable, which leaves experimental models as the only possible option with which to comprehensively compare the early stages of endocrinopathic and SIRS-associated laminitis.

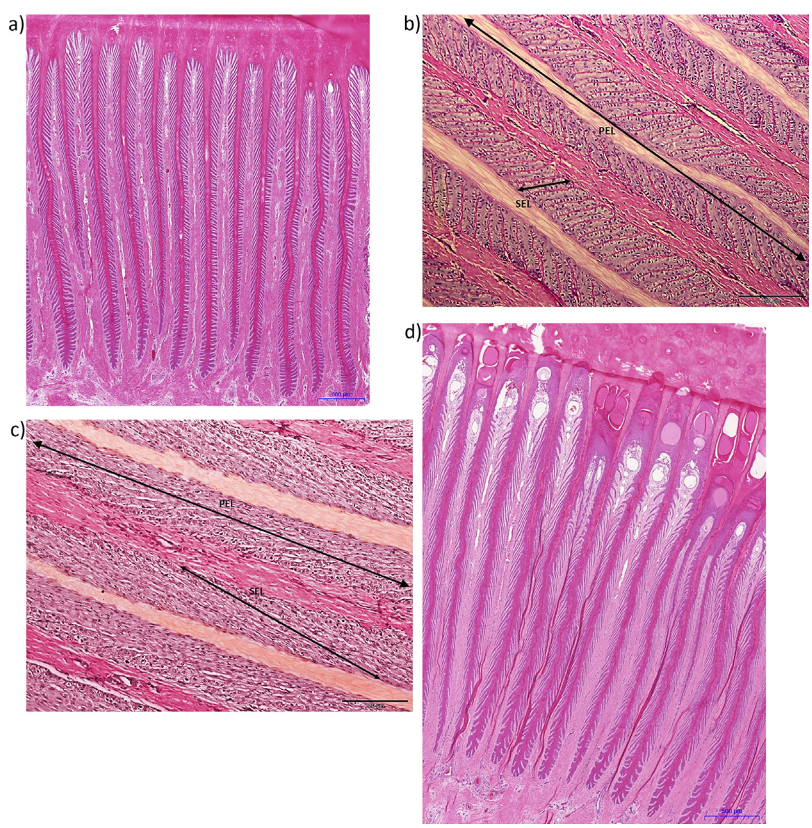

Fig. 2. Normal or 'standard' lamellae of the equine hoof, representing an integrated epidermal and dermal unit (epidermis overlying dermis) at (a) 2.5× original magnification and (b) 200× original magnification. The dermis arises axially from mesenchymal cells overlying the distal phalanx with the epidermis arising from overlying ectodermal cells, which develop and invaginate to create primary and secondary lamellae. (c) In the 200× original magnification of laminitic secondary lamellae, the tips of the secondary epidermal lamellae (SEL), which are acutely angled to the axis of the primary epidermal lamellae (PEL), are lengthened, narrowed and tapered (rather than club-shaped), with irregularity of the PEL/SEL interface. (d) Lamellae showing naturally occurring laminitic changes axially, adjacent to the dermis, at the bottom of the image, to abaxially, adjacent to the hoof wall, at the top of image (2.5× original magnification).

Morphological appearance of the normal lamellae

The normal morphology of the lamellar region is variable in terms of primary and secondary lamellar length, width, orientation and type, which may reflect age, weight and the mechanical environment (Kawasako et al., 2009; Karikoski et al., 2015). Consequently, it can be challenging for the pathologist or researcher to distinguish true pathological changes from normal morphological variation. A classification system for the morphology of primary and secondary epidermal lamellae in 'normal' (35 Thoroughbred cadaver) horses described substantial histological diversity, with hyperplastic secondary epidermal lamellae (SEL) predominating in axial regions independent of clinical signs of laminitis (Kawasako et al., 2009). This finding led to the suggestion that attempts to diagnose or grade the severity of laminitis based on histology would be ill-advised. However, since some of the hooves studied were from horses euthanased for systemic inflammatory disease, subclinical/early SIRS-associated laminitis may have confounded these data. The same classification system was then applied to normal aged (≥12 years) and young (<12 years) horses and ponies that were stringently evaluated using clinical inclusion and exclusion criteria inclusive of endocrine status (Karikoski et al., 2015). Using this approach, lamellar hyperplasia was not detected and the most common epidermal lamellae subtypes were 'standard' (Fig. 2a and b). Primary epidermal lamellae (PEL) were significantly longer in horses than ponies, and in older horses vs. their younger counterparts, both of which were attributed to body weight/cumulative biomechanical force (Karikoski et al., 2015).

Lamellar and cellular stretching is a key early lesion

Documented early histological changes in endocrinopathic and SIRS-associated laminitis models have included loss of the perpendicular orientation of SEL nuclei relative to their basement membranes (BMs), nuclear rounding, a more centrally located nucleus within the cytoplasm (vs. apical), with a more random orientation and prominent nucleoli (Pollitt, 1996; Asplin et al., 2010; de Laat et al., 2011b, 2013a). In both forms of laminitis, SEL were observed to lengthen, narrow, develop tapered (vs. club-shaped) tips and to become more acutely angled to the PEL axis, with irregularity of the PEL/SEL interface. The PEL and SEL were more closely apposed and frequently became difficult to distinguish (Pollitt, 1996; Asplin et al., 2010; de Laat et al., 2011b, 2013a) (Fig. 2c).

SEL elongation was thought to occur partly due to epidermal cells sliding past each other (Pollitt, 2004) or because of increased proliferative activity provoked by insulin (de Laat et al., 2013b). Subsequently, lamellar/cellular elongation was found in fore and hind feet of ponies with experimentally induced hyperinsulinaemic laminitis, in the absence of significant lamellar disruption (Karikoski et al., 2014). Stretching is now regarded as a key early (and potentially primary) structural event, and was the earliest noticeable histological change at the 6 h time point in Standardbred horses with induced hyperinsulinaemia (de Laat et al., 2013a). This cellular stretching was accompanied and followed by evidence of an accelerated cell death-proliferation cycle, although the spatial/temporal relationships of these processes (cell death, proliferation and stretching) remain obscure and vary between models/studies (de Laat et al., 2013a; Karikoski et al., 2014). Lamellar epithelial cell proliferation has not been evaluated in SIRS-associated laminitis, but in hyperinsulinaemic horses it occurs in acute experimental models and in chronic natural cases of laminitis (de Laat et al., 2013a; Karikoski et al., 2014, 2015). Apoptotic cells are a rarity in normal lamellar tissue and their enrichment in hyperinsulinaemic models may be secondary to mechanical stress caused by lamellar epithelial cell stretching (Asplin et al., 2010; Karikoski et al., 2014). Retrospective examination of specimens from multiple hyperinsulinaemia models suggests that it commences axially (adjacent to the distal phalanx), spreading abaxially (toward the hoof wall) in a 'wave' that is followed by proliferation (Asplin et al., 2010; de Laat et al., 2013a).

Is the suffix '-itis' applicable to the endocrinopathic disease?

Endocrinopathic laminitis, unlike SIRS-associated laminitis, is associated with surprisingly minimal (histologically observable) inflammation in both (chronic) naturally occurring and (acute) experimentally induced laminitis, even where acute lamellar tearing occurs (Asplin et al., 2010; de Laat et al., 2011b, 2013a; Karikoski et al., 2014, 2015). In general, the extent and severity of inflammation (indicated by neutrophils and activated macrophages) in both types of laminitis is less than that seen in many other tissues (including skin) subject to similar levels of stress and mechanical compromise (Morgan et al., 2003; Karikoski et al., 2014). However, the more significant leucocyte accumulation noted in lamellar tissue in the developmental phase of carbohydrate overload-induced laminitis, which precedes and then synchronously peaks with epithelial cell stress at the onset of lameness (Black et al., 2006; de Laat et al., 2011b; Faleiros et al., 2011), may indicate a key pathophysiological difference. The term 'laminopathy' might be a more appropriate morphological descriptor for endocrinopathic laminitis.

Re-defining the basement membrane focus

Prior to the development of the hyperinsulinaemic model, the primary lesion of laminitis, and research focus, was generally accepted to be BM injury (Pollitt, 1996; Eades, 2010). Diagrams depicting a 'degloving' injury were based on observations of lamellar explant separation under specific experimental conditions (Pollitt, 1996, 2004), and early pathological changes in SIRS-associated laminitis that implicated multiple BM targets (French and Pollitt, 2004; Wang et al., 2012, 2013).

Interestingly, BM damage was not found in all carbohydrate overload models, with one group noting that it remained intact and attached during the acute phase (Morgan et al., 2003). In most hyperinsulinaemia models, BM damage was minimal and predominantly localised to the most axial SEL only; neither all SEL, nor all specimens were affected (Asplin et al., 2010; de Laat et al., 2011b, 2013a). BM separation was absent ultra-structurally in hyperinsulinaemic ponies (Nourian et al., 2009); there were reduced numbers of hemi-desmosomes per unit length of BM, but this was to a lesser degree than that measured in a carbohydrate overload model (Nourian et al., 2007, 2009). However, in two horses, more severe membrane separation occurred along the length of the affected PEL following 48 h of hyperinsulinaemia, with an observed increase in the severity of clinical signs (de Laat et al., 2011b).

Matrix metalloproteinases (MMPs) are often proposed to be the agents of BM breakdown, although we lack direct evidence for their activities. Multiple MMPs are upregulated in SIRS-associated laminitis with (some) significant variation between individual horses, but only pro-MMP-9 was increased in horses with induced hyperinsulinaemia at 48 h, possibly due to small numbers of infiltrating neutrophils (de Laat et al., 2011a). Furthermore, a failure to demonstrate increased MMP-2 and MMP-9 (gelatinase) activities in severe (carbohydrate-induced) laminitis was attributed to the activities of tissue inhibitors of MMP (Loftus et al., 2009). Therefore, a primary role of BM pathology is debatable for both endocrinopathic and SIRS-associated laminitis, although it undoubtedly plays a (variable) role in the cascade of lesions following disease initiation.

The above findings do not clearly indicate if there is a genuine, qualitative difference in histopathology between SIRS-associated and endocrinopathic laminitis. While it is possible that differences seen in structural/histological lesions in lamellae and their BM could reflect the more precipitous nature of inflammatory disease, the hyperinsulinaemia models also involve abrupt onset. An analysis of the histological changes in strictly defined, naturally occurring cases of endocrinopathic disease was therefore considered to be essential in establishing whether these have any relationship to lesions noted in the acute, experimental scenarios.

Histopathology of naturally occurring endocrinopathic laminitis and the identification of a preclinical stage

The histopathology of naturally occurring endocrinopathic laminitis was described in 14 horses and ponies with reference to 25 age and breed-matched controls (Karikoski et al., 2015). Microscopic lesions were largely localised abaxially (close to the hoof wall) and included apoptosis, lamellar fusion, hyperplasia and partial replacement with aberrant keratin containing nucleated debris and proteinaceous lakes. The abaxial location of continuing apoptosis correlated well with the axial-to-abaxial 'wave' of cell death hypothesised from the developmental phases of experimental models. These abaxial lesions were associated with irregular margins between the stratum medium and the lamellar tissue, and may indicate the following sequential events: (1) fusion of SEL tips across the supporting dermal tissue, isolating rounded areas of vascular dermal tissue; (2) continued centrifugal keratinisation with production of increasing amounts of cap horn; degeneration of blood vessel(s); and (3), infill with keratin or proteinaceous fluid (Karikoski et al. 2015; Fig. 3).

In some horses with endocrinopathic laminitis, evidence of acute tearing was superimposed on chronic pathology. Tearing in chronic endocrinopathic laminitis was always abaxial and variably involved BM separation, tearing of SEL and/or separation of the PEL by ripping through the SEL bases (Karikoski et al., 2015). In acute induction models associated with SIRS, tearing was mostly axial and exclusively involved BM separation (Pollitt, 1996; de Laat et al., 2011b). Axially in lamellar tissue from the endocrinopathic case series, the lesions were less severe, but included tapering of SEL and PEL ends (Fig. 2d). The largely abaxial localisation of chronic lesions is incompletely understood, but may indicate a weakness where SEL are anatomically proximal to each other, i.e. radial arrangement around the abaxial end of primary dermal lamellae (PDL), and experience potentially greater metabolic and/or mechanical stress.

While the lesions seen in these naturally occurring cases are consistent with progression of the lamellar stretching and deformation noted acutely and possibly primarily in hyperinsulinaemic models, they still do not clearly indicate if endocrinopathic and SIRS-associated laminitis are fundamentally different diseases. However, chronically developing endocrinopathic lesions were found to have a preclinical stage, implying a 'window of opportunity' for intervention (Karikoski et al., 2015). This assertion is based on data showing that, while the type/severity of lamellar lesions is not correlated with the reported duration of pain (5–730 days, median 120 days), divergent hoof rings were noted in all but one animal (13/14 horses); these rings would have taken longer to develop (approximately 3 months; Karikoski et al., 2015) than the known duration of laminitis (as a

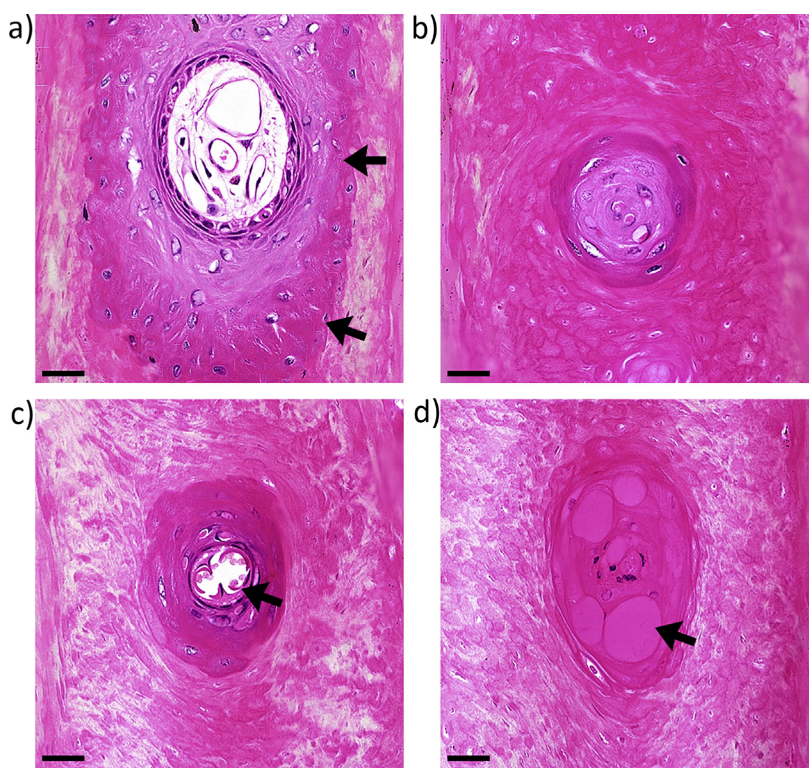

Fig. 3. A range of abaxial lesions noted in horses with naturally occurring hyperinsulinaemic laminitis (often within the same specimen) that were interpreted as representing the following sequence of events: (a) fusion of tips of the secondary epidermal lamellae (SEL) across the primary dermal lamellae (PDL), resulting in isolation of rounded areas of vascular dermal tissue that continue to keratinise peripherally (centrifugally) and produce increasing amounts of cap horn; (b) reduction in the area of this dermal 'circle', with complete isolation from the more axial (epidermal and dermal) tissue by keratin, and keratinisation of most of the immediately surrounding epidermal tissue; (c) degeneration of the blood vessel(s) and completion of keratinisation of the remaining (central) epidermal cells; and (d) filling of the area with keratin or proteinaceous fluid. Rows of these isolated and degenerate/keratinised circular foci were seen in the cap horn in many fields. Scale bar = 25 μm.

clinical sign). These macroscopic changes typically have been associated with previous clinical episodes of laminitis or prolonged chronic laminitis (Pollitt, 2004; Eustace, 2010). Management of endocrine disease at this crucial stage might slow, prevent or even reverse lamellar pathology, with the prevention of lamellar stretching being an attractive therapeutic target in hyperinsulinaemia models.

Lamellar epithelial cell stretching: Possible mechanisms in the hyperinsulinaemic horse

As above, histological findings in models of acute stages of hyperinsulinaemia indicate stretching or elongation of lamellar epithelial cells as a key early event. The (controversial) theory of tensegrity usefully explains the forces that stabilise cells and how these might be eroded in instances of mechanical failure. The cytoskeleton is the arbiter of cell shape and mechanical properties, with microtubules and actin microfilaments serving as compression-resisting elements (struts) and tensile components, respectively; their interactions contribute to a pre-existing intracellular tension termed 'pre-stress' (Ingber et al., 2014). Filamentous actin can organise as filaments or bundles, with small variations in filament or cross-link concentration, or external pre-stress, rapidly altering mechanical strength of the cell (Gardel et al., 2004). The major cytoskeletal proteins in equine lamellar tissue are keratins (intermediate filaments, IFs) that contribute significantly to the mechanical properties of epithelial cells (Seltmann et al., 2013; Carter et al., 2014). They are needed to anchor and exert strain on the nucleus, while stabilising the cytoskeletal network as a whole (Ingber, 2003; Maniotis et al., 1997). The cytoskeleton also intersects with a highly elastic geodesic network of proteins (actin, ankyrin and spectrin) located directly beneath the cell membrane; the efficiency of this coupling depends on cell surface adhesion complexes (i.e. integrins; Ingber, 2003). This theory explains the transmission of local stresses over the cell's entire cytoskeletal architecture. At the higher level of cellular populations, there exists a complex relationship amongst cell shape, pre-stress, connections with other cells, connections to the BM, and the physical properties of that membrane (Ingber and Jamieson, 1985).

The BM is proposed to be a tension element of this higher order tensegrity system, distributing physical forces in addition to attaching epithelial and dermal components to each other (Ingber and Jamieson, 1985). Any alteration to these components could alter epithelial cell pre-stress, triggering events that may be difficult to determine using in vivo equine models. Rapid elongation of the lamellar epithelial cells (within 6 h in the hyperinsulinaemia models) implies plastic deformation of the cytoskeleton, (Douglas et al., 1998; Davies et al., 2007). How or when this might occur in association with more slowly developing hyperinsulinaemia remains unknown. An alteration in keratin expression has been demonstrated in basal and suprabasal cells in the first 48 h of laminitis, although at least one of the three horses had SIRS-associated laminitis (Wattle, 2000).

In carbohydrate overload models, cytokeratin 14 immunofluorescence did not diminish, but became clustered around the nucleus (French and Pollitt, 2004). It is also important to appreciate that the cellular ability to tune pre-stress is essential for homeostatic control, potentially explaining many of the chronic disturbances that accompany the acute phase. At the subcellular level, the nucleus is linked to surface adhesion molecules via the IF network, with transmission of mechanical forces and physical cues cable of influencing the transcriptome (Maniotis et al., 1997; Wang et al., 2009). Collectively, these data suggest that cellular stretch could direct early pharmacological intervention strategies in horses showing preclinical divergent hoof ring formation.

Is stretching of lamellar epithelial cells due to direct actions of insulin?

Prolonged hyperinsulinaemia has not been found to upregulate gene expression of (selected) markers of insulin signalling and glucose transport in lamellar tissues (Campolo et al., 2016). In studies using hoof lamellar explants, glucose uptake was unaffected by insulin. Instead, mechanistic aspects of glucose uptake, in conjunction with the expression of mRNAs for GLUT1 and GLUT4, indicated the dominance of a GLUT1 (insulin-independent) glucose transport system, rather than a glucose deprivation model for laminitis (Asplin et al., 2011). In hyperinsulinaemia models, GLUT1 was unaffected, although there were increases in novel GLUTs (GLUTs 8 and 12; de Laat et al., 2015). Insulin receptors are not expressed by lamellar epithelial cells, but may be located in the vascular/stromal compartment, with gene expression decreasing in hyperinsulinaemia models (Burns et al., 2013; de Laat et al., 2013b). Taken together, these findings do not indicate a direct cellular effect of insulin, but there are significant numbers of insulin-like growth factor (IGF)-1 (tyrosine kinase) receptors (IGF-1Rs) in equine lamellar tissue (Kullmann et al., 2016). While serum IGF-1 concentrations did not changesignificantly over a 46 h period in hyperinsulinaemia models (de Laat et al., 2013b), it is plausible that, at high concentrations, insulin may bind to and activate IGF-1R, leading to receptor down-regulation via negative feedback (de Laat et al., 2013b). Supportive evidence is provided by a report of reduced levels of lamellar IGF-R1 in obese horses, together with significant reductions in acute hyperinsulinaemia models (de Laat et al., 2013b; Kullmann et al., 2016). In other cell types, IGF-1R has been shown to interact in a dynamic fashion with integrins, intercellular adhesion molecules, the cytoskeleton and intracellular signalling cascades, including mitogen-activated protein (MAP) kinases and phosphoinositide 3 (PI3) kinase. Reorganisation of the cytoskeleton can occur in response to receptor activation, largely studied in the context of cancer cell motility, migration and epithelial to mesenchymal transition (EMT; Julien-Grille et al., 2013).

The morphological alterations of lamellar epithelial cells include some aspects of EMT, such as flattening, spreading and loss of cell-cell contacts. However, at least in the carbohydrate overload model, there is no direct evidence of invasiveness/ motility, loss/internalisation of E-cadherin, gain of vimentin expression (Wang et al., 2013) or propensity for malignant transformation. While links exist between GLUT1 and the cytoskeleton, these relate to subcellular targeting rather than remodelling (Bunn et al., 1999). Determining whether there is a direct hormonal influence on cellular cytoskeletal mechanics will likely necessitate in vitro experimentation with superior lamellar cell/explant culture models.

Is it the basement membrane after all?

As already mentioned, lamellar epithelial cell stretching may reflect a hormone-induced change in the pre-stress state. However, in cell culture, cells with fewer surface adhesions manifest reduced stiffness and may rely more on internal microtubules to generate pre-stress (Wang and Ingber, 1994). Conceivably, even minor detachment associated with the subtle BM damage seen in hyperinsulinaemia models could contribute to alterations in epithelial cell tensegrity parameters. The distinction between primary or secondary BM pathology may be very difficult to make; if lamellar epithelial cell elongation is primary, conceivably there would be some change in the BM structure and/or elasticity to accommodate this that would occur within a narrow time frame. Such distinction within narrow time frames might be assisted by detailed scrutiny of BM components, including proteolytic fragments (Visser and Pollitt, 2011), combined with traditional observational approaches.

While it is possible that endocrinopathic laminitis is a (structurally) subtle lamellar BM disease, there is mixed evidence for related inflammatory changes (as indicated in SIRS-associated laminitis). While relationships between obesity-associated diseases and pro-inflammatory cytokines are well established for human patients (Dandona et al., 2004), the scenario in horses is less clear. Pro-inflammatory effects, including increased levels of tumour necrosis factor (TNF)-α, have been related to obesity (Suagee et al., 2011) and have been noted in lamellar tissue of horses with marked hyperinsulinaemia and laminitis (Obel grade 2; de Laat et al., 2014). However, increases in similar mediators were not found in horses with moderate hyperinsulinaemia and subclinical lamellar pathology in the hyperglycaemia model, suggesting that they may not be involved in the earliest/key stages of pathology. Additionally, ponies that were overfed carbohydrate (provoking hyperinsulinaemia) failed to show general up-regulation of lamellar pro-inflammatory cytokine gene expression or leucocyte infiltration (Burns et al., 2015). However, that study reported increased gene expression of cyclooxygenase-2 (COX-2); COX-2 signalling has been linked with IGF-1 stimulation and EMT (Tian et al., 2012). In recent studies of carbohydrate overload models, significant increases in inflammatory gene expression and transforming growth factor (TGF)-β1 protein were found in micro-dissected lamellar basal epithelial cells (Leise et al., 2015; Gutzmann et al., 2016). This type of tissue compartment-specific analysis (proteomic or next generation sequencing [NGS]) is likely to be valuable in further exploration of the possible roles of inflammation and BM damage in endocrinopathic laminitis.

Conclusions

The aim of this review was to highlight recent advances in our understanding of the pathophysiology of endocrinopathic laminitis, with suggestions as to future research avenues. Specifically, laminitis is now considered to be a syndrome that most commonly results from endocrine disease, with evidence of a prolonged subclinical phase in at least some horses, as evidenced by the development of divergent hoof rings. These hoof rings may signify a window of opportunity for therapeutic intervention. Histologically, structural BM lesions are subtle in comparison with previous descriptions of rapid failure in SIRS-associated laminitis, although other lesions are very similar, suggesting convergent mechanisms or limited responses available to a highly specialised tissue. Lamellar epithelial cell stretching is an early and key event, suggesting cytoskeletal deformation and eroded mechano-protection. Further mechanistic research is now warranted to explore any causal relationship(s) between hyperinsulinaemia and these cellular changes. Investigations should focus on: (1) the earliest changes, possible invisible histologically or ultra-structurally; (2) analyses of specific tissue compartments using NGS and/or proteomics, in particular of basal lamellar epithelial cells; and (3) investigation of possible links between insulin, IGF-1R, cellular tensegrity, inflammatory mediators, and BM composition/structure. Basic research at the cellular/molecular level is likely to be necessary to determine precisely how hyperinsulinaemia drives the key and possibly preventable lesion of lamellar stretching.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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